MICROBIAL EXPRESSION OF HUMAN INFLUENZA HEMAGGLUTININ PROTEINS

FIELD OF THE INVENTION

This invention relates to the use of recombinant DNA technology for the microbial production of human influenza hemagglutinin proteins for use in the preparation of vaccines for treating human influenza. In one aspect, the present invention relates to the construction of microbial expression vehicles containing DNA coding for the antigenic determinants of the hemagglutinin gene of human influenza and to the expression vehicles so constructed. In another aspect, the present invention relates to the means and methods for microbially expressing the antigenic determinant(s) of the hemagglutinin gene of human influenza. In yet another aspect, this invention relates to the novel end products of the microbial expression referred to above and to the means and methods of converting such products to entities useful against human influenza.

BACKGROUND OF THE INVENTION

A. Human Influenza Hemagglutinin Proteins

Influenza is a major, acute respiratory disease of human beings. It occurs in recurrent endemic and pandemic infections which start abruptly, spread rapidly and distribute frequently worldwide. Although the disease is usually relatively mild in healthy individuals, its results cause major financial losses from lost time at work and unaccountable impact in terms of pain and suffering. Thus, the prevention of outbreaks of influenza would be of great economic and social value.

The disease is caused by a virus vector which invades and infects host organism cells, disrupting their useful functions. Vaccines for use against influenza, prepared from killed virus, have been in use since the early 1940s. However, the usefulness of these vaccine products has been hampered by several problems such as:

(a) recipients of vaccine innoculations have not always reacted with protective effect,

(b) the potency of such vaccines has been variable from batch to batch and virus type to type,

(c) the administration of (frequently required) large amounts of vaccine produces adverse reactions often exceeding the tolerable limits of the human organism,

(d) their method of production from chick embryos (eggs) can cause incidental toxic effects because of unremovable egg impurities, and

(e) search for a suitable live attenuated influenza virus vaccine has not yet been successful because of possible reversion of such virus to wild type during administration into the human population.

These and other factors influence their widespread use and influenza remains as a dreadful disease and those individuals in the elderly age group and/or those who have chronic physical ailments are often susceptible to a greater degree. Thus, it would be very desirable to have a vaccine product for human influenza which, because of the method by which it is prepared, and its constituency, would overcome these problems.

The hemagglutinin (HA) protein is the most important protein involved in immunity against influenza virus. The hemagglutinin protein occurs as glycoprotein spikes on the virus surface. It has been shown to be structurally triangular and rod-shaped and is comprised of several subunits which contain the major antigenic and immunogenic determinants. The antigenic determinants are the antigenic binding sites for specific antibodies. The stimulated production of specific antibodies produces a state within the host organism of immunity to viruses containing the same antigenic determinants. Once induced, these antibodies can remain in the host organism for a significant period of time and later their production can be readily stimulated by reimmunization.

The various subunits of the hemaggluttinin protein are synthesized as part of a single polypeptide chain containing an amino acid precursor peptide attached at the N-terminus of the overall HA protein. Studies have shown that one of these units, referred to as HA1, which is located at the N-terminus of the uncleaved HA is the primary area of the HA molecule which contains the major antigenic determinants. There are a large number of strains and types of influenza viruses. They are distinct from one another by virtue of possessing variations in the antigenic determinants. These variations are referred to as "shifts" and "drifts" depending upon the extent of genetic variation. Thus, antibodies induced from one strain or type do not necessarily protect the host from a different strain. The variations in the antigenic determinants, referred to above, are due to mutations as well as reassortment in the viral genome that in turn lead to amino acid substitutions within the antigenic sites of the HA protein.

Reference is made to *Structure and Variation in Influenza Virus*, Proceedings of the International Workshop on Structure and Variation in Influenza Virus, Thredbo, Australia, Dec. 10–12, 1979, published by Elsevier North Holland, Inc., New York, N.Y., 1980, Editors: Graeme Laver and Gillian Air, in order to further illuminate the background of the present invention and to provide additional detail respecting its practice. By this reference, this citation is hereby incorporated herein.

Thus, it would be desirable to produce vaccines which can be readily modified to account for naturally occurring changes in the various viral strains, by focusing on the hemagglutinin protein itself, determining its, or at least its antigenic determinants', sequence(s) and preparing a vaccine containing the new or changed protein. The present mention provides the methods and means therefor.

B. Recombinant DNA Technology

With the advent of recombinant DNA technology, the controlled bacterial production of useful polypeptides has become possible. The workhorse of recombinant DNA technology is the plasmid, an extrachromosomal loop of double-stranded DNA found in bacteria, oftentimes in multiple copies per bacterial cell. Included in the information encoded in the plasmid DNA is that required to reproduce the plasmid in daughter cells (i.e., a "replicon") and ordinarily, one or more selection characteristics, such as resistance to antibiotics, which permit clones of the host cell containing the plasmid of interest to be recognized and preferentially grown in selective media. The utility of plasmids, which can be recovered and isolated from the host microorganism, lies in the fact that they can be specifically cleaved by one or another restriction endonuclease or "restriction enzyme," each of which recognizes a different site on the plasmidic DNA. Thereafter heterologous genes or gene fragments may be inserted into the plasmid by endwise joining at the cleavage site or at reconstructed ends adjacent to the cleavage site.